CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION

MEDICAL TOXICOLOGY BRANCH
SUMMARY OF TOXICOLOGY DATA

BROMOXYNIL OCTANOATE, BROMOXYNIL

Chemical Code 834, Document Processing Number (DPN) 324 and 50687
SB 950 No. 025 and 542

June 8, 1987
Revised: Jan. 26, 1988; Nov. 4, 1988; Feb. 22, 1989;Dec. 19, 1989; June 6, 1990; Nov. 18, 1991; Jan. 24, 1992; Feb. 4, 1994; March 10, 1995; and August 11, 2005

I. DATA GAP STATUS

Combined (chronic + onco): No data gap, possible adverse effect indicated

Chronic dog: No data gap, possible adverse effect indicated (in subchronic study[1])

Onco mouse: No data gap, possible adverse effect indicated

Repro rat: No data gap, possible adverse effect indicated

Terato rat: No data gap, possible adverse effect indicated

Terato rabbit: No data gap, possible adverse effect indicated

Gene mutation: No data gap, possible adverse effect indicated

Chromosome: No data gap, possible adverse effect indicated

DNA damage: No data gap, possible adverse effect indicated

Neurotox: Not required (not an organophosphate)

---

[1] Cataracts were induced in dogs after oral exposure to Bromoxynil Octanoate for 13 weeks (record 139461 [p. 5]). There are no long-term oral studies of Bromoxynil Octanoate in dogs on file with DPR.

Note: Toxicology one-liners are attached.
****** indicates acceptable study (see note at top of page 2 regarding the reclassification of many previously unaccepted studies to acceptable)
Bold face indicates possible adverse effect.
File name: T050811
Revised August 11, 2005 by S. Rinkus
These pages contain summaries only. Individual worksheets should be reviewed as they may

contain additional effects.
DPN# 324 = Bromoxynil Octanoate; DPN# 50687 = Bromoxynil Butyrate
Note: Bromoxynil (unesterified), Sodium Bromoxynil, and Bromoxynil Butyrate are not registered for use in California

II. TOXICOLOGY SUMMARY: BROMOXYNIL OCTANOATE STUDIES

It is the Medical Toxicology Branch's opinion that a question of equivalency between Bromoxynil and Bromoxynil Octanoate exists, based on evidence that the two chemicals have different pharmacokinetics, toxicological effects and potencies. However, a decision has been made by the Department of Pesticide Regulation (12/23/94) to accept the conclusion of the USEPA that Bromoxynil and Bromoxynil Octanoate are equivalent for toxicological testing. A copy of a memorandum of the USEPA, dated December 5, 1994, DP Barcode D209726, from Edwin Budd, Toxicologist, HED, to Kathryn Davis/Thomas Luminello, Jr., Chemical Review Manager Team 52, regarding the "bridging studies" of Bromoxynil and Bromoxynil Octanoate was received by the Department of Pesticide Regulation. The decision of the USEPA is stated on page 3 of the document as "...the compounds Bromoxynil phenol and Bromoxynil octanoate may be considered to be toxicologically equivalent" (underlining as in the USEPA document). Based on the decision to defer to the USEPA, the toxicological studies for Bromoxynil and Bromoxynil Octanoate are now considered grouped in terms of fulfilling data requirements under SB 950 at this time. (Rinkus, 3/10/95).

CHRONIC TOXICITY, RAT

No studies on file.

SUBCHRONIC TOXICITY, RAT

324-024 940481 Title: Chronic Toxicity of M&B 10731 (Bromoxynil octanoate) (Technical grade) in the rat (13 week subchronic oral). (Huntingdon Research Centre, 5-10-65, Report No. 1222/65/139). Bromoxynil octanoate technical grade powder (no purity stated); doses of 0, 20, 50, 125, 312, 781 or 1953 ppm fed in the diet to groups of 10/sex Sprague-Dawley rats per group with 30/sex in controls, for 13 weeks. Increased kidney and liver weights at 781 and 1953 ppm in males and females, changed blood profile at 1953 ppm. Supplemental to chronic studies. Reviewed by JR(G), 3-11-85. The absolute weight of the thyroid and pituitary were decreased in the males; the respective LOELs were 125 ppm and 312 ppm. Paradoxically, increased absolute thyroid weight was seen in the 20 ppm and 50 ppm female groups and increased absolute pituitary weight was seen in the 50 ppm female group. Since an increasing dose response was not seen, both of these increases may be spurious. However, the magnitude of the difference from the control values was impressive and may be indicative of a significant biological effect. (Rinkus, 3/10/95).

324-010, -023 940477, 940476 "Herbicides: Bromoxynil Octanoate--M&B 10,731. Histopathological Study of Rat Tissues from 90-day Subacute Experiments Completed at the Huntingdon Research Centre," (Neville Woolf; May & Baker Ltd., Dagenham, Essex; Report No. RPAD.VISIT.135; August, 1965). This record contains histopathology data for 5 rats/sex that were administered 0 and 1953 ppm Bromoxynil Octanoate in the diet in record 940481. **Supplemental information; no worksheet.** (Rinkus, 3/10/95).

324-140 116895 "13-Week Dietary Toxicity Study with Bromoxynil Octanoate in Rats" (Hazleton Wisconsin, Inc.; Project No. HWI 6224-170; 6/25/92). Bromoxynil Octanoate (lot no. CN51032; 95.2% purity) was administered in the diet at 0, 150, 600, 1100 and 2100 ppm to 20-30 Crl:CD*BR VAF/Plus* rats/sex/group. Analytical studies indicated that Bromoxynil Octanoate was unstable in the feed and that degradation to Bromoxynil accounted for only 34-40% of the loss of test material. Due to the instability, starting after study week 3, the weekly preparation of diets was stored frozen and fed to the rats four times per week. The only deaths in the study occurred in the groups given the 2100 ppm diet. Twenty six females and nine males in the 2100 ppm groups (30/sex) were found dead, mainly on study days 3-5. Most of the rats found dead did not exhibit any clinical signs that would be suggestive of their impending deaths. The survivors in the 2100 ppm groups were sacrificed on study day 9. Each of the survivors had lost 6-34 grams in bodyweight during the first study week. Other findings for the survivors (both sexes) included: greatly decreased platelet count; increased RBC count, hemoglobin concentration and hematocrit; increased blood urea nitrogen, SGOT and SGPT; decreased serum globulin; lymphocytic necrosis and degeneration in the spleen, thymus, and mesenteric lymph node; hematopoietic depletion in the bone marrow; and stomach hemorrhages and congestion. Bodyweight effects also were seen in the lower dose groups: the 600 ppm and 1100 ppm females had mean BWs during study weeks 2-14 that were 90-94% and 78-81% of the control values, respectively; the 1100 ppm males had mean BWs during study weeks 2-14 that were 84-87% of the control values. These BW effects were not accompanied by a decrease in food consumption, except for the first study week. The 1100 ppm female group exhibited the following hema-tological effects: decreased platelet count and increased RBC count, hemoglo- bin concentration and hematocrit in the testing done at study week 5; and an increased lymphocyte count in the testing done at study week 14. The following clinical chemistry effects were seen at study week 5, but not study week 14: an increase in serum albumin in the 150 ppm and 600 ppm female groups and in the 600 ppm male group; decreased total bilirubin in the 600 ppm and 1100 ppm female groups; decreased serum potassium in the 1100 ppm female group; decreased serum chloride in the 600 ppm and 1100 ppm female groups. The following clinical chemistry effects were seen at study weeks 5 and 14: a decrease in total serum protein in the 1100 ppm groups (both sexes); a decrease in the globulin concentration in the 1100 ppm groups (both sexes). At study week 14, the 1100 ppm groups (both sexes) also exhibited an increase in serum alkaline phosphatase. No effects on blood urea nitrogen, SGOT or SGPT were noted in the testing done at study weeks 5 and 14. Increased absolute liver weight and decreased absolute right adrenal weight were seen in the 600 and 1100 ppm female groups; the adrenal weight reductions were not statistically significant when viewed relative to bodyweight, although the decreases were still apparent. Organ weight was not determined for the heart, pituitary or thyroid. Myocardial degeneration and necrosis were observed in both sexes. The incidence rate in the males was: 0 ppm, 5/30; 150 ppm, 7/20; 600 ppm, 8/20 ($p = 0.066$); and 1100 ppm, 11/20 ($p = 0.006$). The

incidence rate in the females was: 0 ppm, 3/30; 150 ppm, 3/20; 600 ppm, 3/20; and 1100 ppm, 10/20 (p = 0.002). Myocardial lesions also were noted in two 2100 ppm females that were found dead on study days 3 and 4. Retinal degeneration was seen in two 1100 ppm males (10% incidence rate); given the rarity of this lesion in rats of this age, this may be an incipient effect. **NOAEL = 150 ppm (myocardial degeneration and necrosis in males, liver hypertrophy in females). Supplemental information.** (Rinkus, 2/22/95).

SUBACUTE TOXICITY, RABBIT

324-085 055498 "Subchronic Dermal Toxicity Study of Bromoxynil Octanoate in Rabbits" (Weaver, E.V. & Homan, E.R.; Bushy Run Research Center, Export, PA; Project Report 46-81; 7/12/83). Bromoxynil Octanoate (technical grade) was applied neat to the backs of 8-10 New Zealand White rabbits/sex/group, at 0, 250, 500 and 1000 mg/kg, for 6 h/day, 5 days/week, for 3 weeks. Individual treatment groups were subdivided so that treatments were applied to 4-5 rabbits/sex with abraded skin and 4-5 rabbits/sex with intact skin. The area of exposure was 4 x 4 inches (10 x 10 cm), which is much less than 10% of the total body surface area for adult New Zealand White rabbits. The test material was applied to a gauze patch and the gauze was affixed to the skin with tape and securely wrapped with polyethylene sheeting. **Supplemental information; no worksheet.** (Rinkus, 3/10/95).

324-138 115692 "3-Week Dermal Toxicity Study with Bromoxynil Octanoate in Rabbits" (Henwood, S.M.; Hazleton Wisconsin, Inc.; Laboratory Project ID: HWI 6224-168; 6/4/92). Bromoxynil Octanoate was applied neat to the backs of 10 Hra:(NZW)SPF rabbits/sex/group, at 0 (water), 30, 300 and 1000 mg/kg, for 6 h/day, 5 days/week, for 3 weeks. The area of exposure was ~10% of the total body surface area (actual dimensions were not provided); and was covered during the 6-h exposure period. **Supplemental information; no worksheet.** (Rinkus, 12/18/92).

CHRONIC TOXICITY, DOG

No studies on file.

SUBCHRONIC TOXICITY, DOG

324-152 139461 "Bromoxynil Octanoate: Toxicity to Dogs by Repeated Oral Administration for 13 weeks," ([author[s] not identified; Huntingdon Research Centre, Huntingdon, England; study number RNP 416/930670; May 27, 1993). Bromoxynil Octanoate (>92% purity), in gelatin capsules without any vehicle, was administered to beagle dogs (two/sex/dose) 7 days/week for 13 weeks. Doses were 0, 0.43, 1.43 and 7.14 mg/kg/day; these represented doses of 0, 1.1, 3.5 and 17.7 µmoles/kg/day, respectively, and were equivalent to doses of unesterified Bromoxynil of 0, 0.3, 1.1 and 4.9 mg/kg/day, respectively. The following discussion of possible treatment-related effects was not based on statistical analyses of the data separated by the sexes; none were supplied in the report and none were conducted in this review, due to the small group sizes. The reporting of clinical signs occurring in the study was inadequate in terms of allowing an independent evaluation. From the limited information that was provided, there was no evidence of a clinical sign being treatment-related. There was no substantial evidence of a treatment-related effect on rectal temperature or food consumption or at hematology or urinalysis. Body-weight gain was reduced in a dose-response fashion in both sexes, starting with the low dose. At the ophthalmology conducted at week 13, five animals were observed to have lens opacities, described as pinpoint or streak (one case); the affected animals came from the low- (one female), mid- (one/sex), and high-dose groups (one/sex). Although the lens opacities constituted gross lesions, they were not examined histologically. Given the young age of the animals, the lack of lens opacities at pretest and the short duration of exposures, the data indicate that the cataractogenesis was treatment-related. Serum-chemistry findings were limited to reduced alkaline phosphatase in the high-dose females (weeks 6 and 13) and, possibly, reduced serum inorganic phosphorus (presumably phosphate) in the high-dose males (week 6). The data for absolute and (or) relative weights indicated that the following organs were affected: liver (increased relative weight at the high dose [males]); kidneys (increased relative weight starting with the low dose [females] or the mid dose [males]); adrenals (increased absolute and relative weights starting with the low dose [males]); and ovaries (reduced absolute and relative weights at the mid dose). Although no unequivocal treatment-related effects were observed at necropsy or histology, findings that warrant concern given the short duration of testing include the following: both high-dose males were diagnosed with inguinal hernias (neither were examined at necropsy or histology); and a red area at the base of the aortic valve in the heart was observed at necropsy in a low-dose male and a mid-dose male (given the same location, the same description at necropsy, and similar sizes for the areas involved, it is not clear whether the different histological descriptions given in the report [hemocyst vs. edema of the inner aortic media] pertain to the same pathological process). Comparison of the effects noted in this study to those noted in the Bromoxynil 13-week and 52-week dog studies (records 139458 and 069883) would indicate that oral exposure to Bromoxynil Octanoate is not categorically equivalent to oral exposure to unesterified Bromoxynil. In order to complete the review of this study, full details of the clinical signs and historical negative-control data for the conducting laboratory need to be submitted and the issues raised in worksheet W139461 821 regarding unsectioned swellings, unexplored hernias, peliosis hepatis and the heart-valve lesions need to be explained. **NOEL < 0.43 mg/kg/day (reduced body-weight gain, cataractogenesis, increased absolute and relative adrenal weights). Supplemental information. (Rinkus, 1/6/05)**.

324-024 940482 Title: Chronic Toxicity of M&B 10731 (Bromoxynil octanoate) (Technical grade) in the dog: (Chronic feeding). (Huntington Research Centre, 2/15/65, Report No. 1177/65/94:3) Bromoxynil octanoate, technical grade, no purity stated; doses of 0, 1, 5 or 25 mg/kg/day given to groups of 3/sex/dose by gelatin capsule for 13 weeks to pedigree dogs (breed not stated.) Some hematological parameters decreased, urea elevated at 25 mg/kg/day. Supplemental information. Reviewed by JR(G), 3-11-85.

324-010, -023 940478, 940475 "Herbicides: Bromoxynil Octanoate--M&B 10,731. Histopathological Study of Dog Tissues from 90-day Subacute Experiments Completed at the Huntingdon Research Centre," (Neville Woolf; May & Baker Ltd., Dagenham, Essex; Report No. RPAD.VISIT.134; August, 1965). This record contains histopathology data for 3 dogs/sex that were given Bromoxynil Octanoate at 0, 5, and 25 mg/kg in record 940482. **Supplemental information; no worksheet.** (Rinkus, 3/10/95).

ONCOGENICITY, RAT

No studies on file.

ONCOGENICITY, MOUSE

No studies on file.

REPRODUCTION, RAT

324-118 095292 "Male Reproductive Effects of Bromoxynil Octanoate after Dermal Administration," (Christian, M.S., Schaeffer, L.A. & Hoberman, A.M.; Argus Research Laboratories, PA; Argus protocol 218-010; 10/19/90). Buctril, containing 33.8% Bromoxynil Octanoate (AI), organic solvents and surfactants, was applied topically to the backs of 125 male Sprague-Dawley rats/group, at 0 (water), 0 (blank), 25, 50, and 100 mg AI/kg/day, for 21 consecutive days. The "blank" group was treated with a solution said to contain the same level of solvents and surfactants as found in the dosing solution for the 25 mg AI/kg group. However, this may not be accurate because skin reactions presumably caused by the solvents were obviously more severe in the blank group than in either the 25 or 50 mg AI/kg groups. Each treatment group consisted of 8 subgroups. One subgroup having 20 males underwent 7 mating trials with un-treated females on the following postdosage days: days 1, 7, 14, 21, 35, 56, and 113; on postdoasage day 119, these males were sacrificed. The seven other subgroups, each having 15 males, were sacrificed sequentially on the postdosage days when the mating trials were initiated with the subgroup containing 20 males. Aside from skin reactions, the only effects observed were changes in liver weight (increased organ-to-BW ratio on postdosing days 1 & 7)

and prostate weight (decreased absolute & relative weights on postdosing day 1): **NOEL = 50 mg AI/kg**. No effects were observed on the following: mating & fertility indices, pre- and postimplantation loss, litter size, sperm count, sperm morphology, sperm motility, the histology of the seminiferous tubules, the epididymis & liver, and the absolute and relative weights of the various male reproductive organs. Deficiencies in this study include: not a dermal FIFRA-834 study; no content analyses of AI or solvents; only males tested; no testing up to a MTD; and application site not covered and much less than 10% of the total body surface area. **Supplemental information.** (Rinkus, 11/22/91).

TERATOLOGY, RAT

Note: The open literature contains two publications regarding oral-exposure, developmental-toxicity testing of Bromoxynil Octanoate and Bromoxynil. In Rogers et al. ("Developmental Toxicity of Bromoxynil in Mice and Rats," Fund. Appl. Toxicol. 17:442-447, 1991), Bromoxynil Octanoate was tested using rats while Bromoxynil was tested using mice and rats. In Chernoff et al. ("Significance of Supernumerary Ribs in Rodent Developmental Toxicity Studies: Postnatal Persistence in Rats and Mice," ibid., p. 448-453), the postnatal fate of supernumerary ribs induced by Bromoxynil in mice versus rats was studied. Full reports of these two studies have not been submitted to DPR (note: the submission of these to DPR should include all of the individual data). (Rinkus, 8/11/05).

324-102 071539 "Dosage Range Developmental Toxicity (Embryo-Fetal Toxicity and Teratogenic Potential) Study of Bromoxynil Octanoate Administered Percutaneously to Cr1:CD(SD)BR Presumed Pregnant Rats." (Argus Research Laboratories, PA; Argus protocol 218-005P; 12/8/88). Buctril, containing 33.8% Bromoxynil Octanoate (AI), solvent, and surfactants, was applied topically to the backs of 8 mated Sprague-Dawley rats/group, at 0 ("vehicle" control), 1, 2, 5, 10, 20, 40, and 100 mg AI/kg/day, on days 6-15 of gestation, with sacrifice on day 20. The "vehicle" control contained the same level of solvent and surfactants as found in the dosing solution for HDT. Maternal toxicity due to the "vehicle" included appreciable skin irritation and decreased bodyweight gains on days 6-12. Fetal effects due to the "vehicle" included decreased fetal bodyweights, delayed sternal ossification, and increased incidence of thoracic ribs. Consequently, maternal and fetal effects due **only** to the AI had to be deduced as those superimposed on the toxicities observed in the "vehicle" control. The slight differences in the maternal weight gains for days 6-12 of gestation between the "vehicle" control and the HDT are of questionable significance. An increased incidence of thoracic ribs and the occurrence of cervical ribs appear to be related to treatments with the AI. Maternal and developmental NOELs only can be calculated for Buctril per se; both NOELs = Buctril diluted with water such that the dose of its AI is 5 mg/kg/day, based on erythema (maternal effect) and extra thoracic ribs (developmental effect). Supplemental information: pilot study. (Rinkus 1/25/89).

324-107 075250 "Developmental Toxicity (Embryo-Fetal Toxicity and Teratogenic Potential) Study of Bromoxynil Octanoate Administered Percutaneously to Cr1:CD(SD)BR Presumed Pregnant Rats." (Argus Research Laboratories, PA; Argus protocol 218-005; 7/3/89). Buctril, containing 33.8% Bromoxynil Octanoate (AI), solvent, and surfactants, was applied topically to the backs of 18-24 pregnant Sprague-Dawley rats/group, at 0 (water), 0 ("blank"), 2, 5, 10, 15, 20, and 75 mg AI/kg/day, on days 6-15 of gestation, with sacrifice on day 20. The water and "blank" controls consisted of two groups each; the groups within a pair differed in age by 2 weeks. The "blank" was said to contain the same level of solvent and surfactants found in the diluted Buctril solution used to treat the 10 mg/kg/day group. However, this may not be accurate, given that 14 of the 50 rats treated with "blank" developed skin irritation while no rats, except those in the 75 mg/kg/day group, developed any skin irritation. Other maternal effects were limited to the 75 mg/kg/day group and consisted of lower bodyweight changes on gestational days 6 and 7 and a lower gestational day 20 corrected bodyweight (day 20 BW minus the gravid uterine weight). **Maternal NOEL = Buctril diluted with water such that the dose of its AI is 20 mg/kg/day (lower BW gain).** Fetal effects included lower bodyweights and an increased incidence of cervical ribs in the 75 mg/kg/day group and a dose-dependent increase in the incidence of extra thoracic ribs, starting with the 15 mg/kg/day group. **Developmental NOEL = Buctril diluted with water such that the dose of its AI is 10 mg/kg/day (extra thoracic ribs).** Major deficiencies included: 1) unclear descriptions of the actual test article and the preparation of the dosing solutions; 2) skin site was not covered even though the volatile organic solvents in Buctril are suscepted teratogens; 3) skin site was much less than 10% of body surface area; and 4) statistical analyses were not done on pooled control fetal data. **Supplemental information.** (Rinkus 9/22/89).

324-105 074446 Draft copy of data tables to record 075250. No worksheet. (Rinkus, 10/26/89).

TERATOLOGY, RABBIT

324-089 085133 "Dosage-Range Developmental Toxicity (Embryo-Fetal Toxicity and Teratogenic Potential) Study of Bromoxynil Octanoate Administered Percutaneously to New Zealand White Rabbits." (Argus Research Laboratories, PA; Argus protocol 218-006P; 9/28/89). Buctril, containing 33.8% Bromoxynil Octanoate (AI), solvent, and surfactants, was applied topically to the backs of 6 inseminated does/group, at 0 ("blank"), 1, 2, 5, 10, 20, 40, and 100 mg AI/kg/day, on days 6-18 of presumed gestation, with sacrifice on day 29. The "blank" was said to contain the same level of solvent and surfactants found in the dosing solution used to treat the 10 mg AI/kg/day group. However, this may not be accurate given that skin irritation was more severe in the Blank group than in the 10 mg AI/kg/day group. Other maternal effects were limited to the HDT and consisted of a lower day 29 BW when corrected for the gravid uterine weight. **Maternal NOAEL = Buctril diluted with water such that its AI is 10 mg/kg/day (severe skin irritation).** No obviously treatment-related effects were observed in the fetuses, but a thorough soft-tissue examination was not performed. **Developmental NOEL = Buctril diluted with water such that its AI is $\geq$100 mg/kg/day (no obvious fetal effects).** Major deficiencies in the study include: 1) unclear descriptions of the actual test article and the

preparation of the dosing solutions; 2) skin site was not covered even though the volatile organic solvents in Buctril are suscepted teratogens; 3) the area of the application site may not have been 10% of the total body surface area; and 4) a true negative control group was not included in the testing. Supplemental information: pilot study. (Rinkus, 11/14/89).

324-112 086712 "Developmental Toxicity (Embryo-Fetal Toxicity and Teratogenic Potential) Study of Bromoxynil Octanoate Administered Percutaneously to New Zealand White Rabbits." (Hoberman, A.M., Argus Research Laboratories, PA; Argus protocol 218-006; 4/27/90). Buctril, containing 33.8% Bromoxynil Octanoate (AI), solvent, and surfactants, was applied topically to the backs of 20 inseminated rabbits per group, at 0 (water; 2 groups), 0 ("Blank;" 2 groups), 5, 10, 15, 20, 40 and 80 mg AI/kg/day, on days 6-18 of presumed gestation, with sacrifice on day 29. The "Blank" was not clearly identified; it was said to contain the same level of solvent and surfactants found in the dosing solution used to treat either the 5 or 10 mg AI/kg/day group. However, even this may not be accurate given that skin irritation was more severe in the Blank group than in either the 5 or 10 mg AI/kg/day groups. No other maternal effects were clearly related to treatments. **Maternal NOAEL = Buctril diluted with water such that its AI is 10 mg/kg/day (severe skin irritation).** The only effects observed in the fetuses were increased incidences of the following skeletal alterations: extra thoracic vertebrae, irregular frontal sutures, and angulated hyoid wings. The absence of clear dose responses for these effects raises the possibilities that they are not related to treatments or that they are somehow related to the excipient in Buctril. Regarding the latter, the possible loss of the organic solvents of the excipient at varying degrees might explain the lack of clear dose responses. A **Developmental NOEL** was not determined in this review due to the need to reanalyze the skeletal alteration data by testing phase. Major deficiencies in the study include: 1) unclear descriptions of the actual test article and of the test material applied to the excipient controls (Blank groups); 2) the skin site was not covered even though the volatile organic solvents in Buctril are suscepted teratogens; and 3) the data were not analyzed by testing phase even though there were definite indications of a testing-phase effect. Supplemental information. (Rinkus, 5/30/90).

324-116 088738 "Recent Increases in the Incidences of Skull, Lung and Rib Al-terations in Vehicle Control New Zealand White Rabbits," (Christian, M.S. et al., J. American College Toxicol. 6:562, 1987). This record is an abstract of a presentation made by these researchers from Argus Research Labs, Inc., at the 7th Annual Meeting of the American College. This apparently is reference 18 that is cited on p. 47 of record 086712. **Supplemental Information. No worksheet.** (Rinkus, 1/24/92).

GENOTOXICITY, GENE MUTATION

Note: Records 001359 and 098204 indicate that the deesterification product, Bromoxynil, is mutagenic in mouse lymphoma cells and Chinese hamster ovary cells in the presence of a metabolic activation system. (Rinkus, 1/24/92).

324-130 098196 "Bromoxynil Octanoate Technical: Testing for Mutagenic Activity with Salmonella typhimurium TA 1535, TA 1537, TA 1538, TA 98 and TA 100," (Dillon, D.M et al.; Inveresk Research International, Tranent, Scotland; IRI Project No. 751126; Report No. 6877; 2/18/91). Bromoxynil Octanoate, technical grade (purity 96.4%) was tested for mutagenicity in the Ames test, using strains TA1535, TA1537, TA1538, TA98 and TA100, in the absence and presence of a metabolic activation system (S-9 made from Aroclor 1254-induced male F344 rat liver). Three separate experiments were performed: first with strains TA1535, TA1537, TA1538 and TA100; second with strains TA1535, TA1537, TA1538 and TA98; and third with strains TA98 and TA100. Testing involved triplicate plating for each treatment level. In each experiment, the test concentrations were: 0 (dimethyl sulfoxide), 0.03, 0.1, 0.3, 1.0, 3.3 & 10.0 mg/plate. Precipitation was consistently noted with the highest treatment level. **No mutagenicity was observed with the test material and the expected results were observed with the positive controls for the tester strains and the metabolic activation system. This study is considered **ACCEPTABLE**. (Rinkus, 11/27/91).

324-149 130788 "Bromoxynil Octanoate, Batch No. HN 81536: Testing for Mutagenic Activity with Salmonella typhimurium TA 1535, TA 1537, TA 98 and TA 100," (Dillon, D.M.; Inveresk Research International; IRI report no. 9904; 10/28/93). Bromoxynil Octanoate (purity 96.0%) was tested for mutagenicity in the Ames test, using strains TA1535, TA1537, TA1538, TA98 and TA100, in the absence and presence of a metabolic activation system (S-9, made from Aroclor 1254-induced male F344 rat liver). Two experiments were performed, involving three platings for each treatment level. The test concentrations were: 0 (dimethyl sulfoxide), 33, 100, 333, 1000, 3333 and 10,000 µg/plate. Precipitation of the test substance occurred at the highest treatment level. The lowest treatment level at which toxicity was observed was 3333 µg/plate with TA1537 when testing in the presence of the metabolic activation system; no toxicity was seen in any strain in the testing done in the absence of the metabolic activation system. **No mutagenicity was observed with the test substance and the expected results were observed with the positive controls for the tester strains and the metabolic activation system. This study is considered **ACCEPTABLE**. (Rinkus, 9/14/94).

GENOTOXICITY, CHROMOSOMAL

Note: Record 001357 indicates that the deesterification product, Bromoxynil, is clastogenic in vitro in the presence of a metabolic activation system. (Rinkus, 1/24/92).

**324-130 098201 "Bromoxynil Octanoate: Micronucleus Test in Bone Marrow of CD-1 Mice," (Holmstrom, L.M & Innes, D.C.; Inveresk Research International, Tranent, Scotland; IRI Project No. 751105; Report No. 6947; 4/11/91). Bromoxynil Octanoate (96.4% purity) was tested for the induction of micronuclei in bone-marrow polychromatic erythrocytes of CD-1 mice of both sexes. Testing involved one-time gavaging of 5 mice/sex/treatment level and sacrificing them at either 24, 48 or 72 hours later. Treatment levels were sex-dependent: males were treated at 0 (corn oil), 52, 105 and 183 mg/kg and females were treated at 0 (corn oil), 76, 153 and 267 mg/kg. Sacrificing at 48 and 72 hours post-dosing was only done with the highest

doses tested. The selection of the high doses was based on LD50 data that were contained in the report. **No induction of micronuclei was observed** whereas the negative control and positive control (cyclophosphamide, 80 mg/kg) gave appropriate results. This study is considered **ACCEPTABLE**. (Rinkus, 12/4/91).

GENOTOXICITY, DNA DAMAGE/OTHER

Note: Record 001358 indicates that the deesterification product, Bromoxynil, inhibited the growth of the DNA-repair-deficient E. coli strain pol A- more so than that of the DNA-repair-proficient strain pol A+, both in the presence and in the absence of a metabolic activation system. (Rinkus, 1/24/92).

324-133 112600 "Bromoxynil Octanoate Technical: Assessment of Genotoxicity in an Unscheduled DNA Synthesis Assay Using Adult Rat Hepatocyte Primary Cultures," (R. Mohammed et al.; Inveresk Research International, Tranent, Scotland; IRI Project No. 751110; Report No. 8121;7/5/91). Bromoxynil octanoate, 96.4% w/w purity, was tested at 0 (DMSO), 0.98, 1.95, 3.91, 7.81, 15.63, 31.25, 62.5 and 125 μg/ml for its ability to induce unscheduled DNA synthesis (UDS) in primary hepatocytes isolated from male F344 rats. Cells were exposed for 18 to 20 hours to test material; UDS was determined using the audioradiographic method. Two independent assays were performed; three experimental units (defined as wells containing a coverslip to which cells had attached) were used per treatment level in each assay. Vehicle controls and positive controls (2-acetylaminofluorene tested at 0.5 and 2.0 μg/ml) included in each assay indicated that the assays were functional. No indication of UDS was observed with Bromoxynil Octanoate up to a concentration of 15.63 μg/ml; at this concentration, relative survival was ≥ 85%. When first reviewed (12/1/92), this study was considered unacceptable but upgradable upon submission of UDS data and/or viability data for the three highest treatment levels. In response, the Registrant submitted record 130844 and this is discussed in worksheet W112600.S01. This study is now considered **ACCEPTABLE. (Rinkus, 9/15/94).

324-150 130844 This record contains an amendment to the UDS study contained in record 112600. The nature of the amendment was to indicate that 0% viable cells (assay not specified) were observed at dose levels of ≥ 31.25 μg/ml. **Supplemental information**. **No worksheet**. (Rinkus, 3/10/95).

NEUROTOXICITY

Not required at this time (not an organophosphate). See last page regarding neurotoxicity concerns for Bromoxynil. (Rinkus, 3/10/95).

RADIOLABEL AND OTHER METABOLISM STUDIES

Note: Records 055496, 055497 and 125665 are studies that used radiolabelled Bromoxynil Octanoate. These studies indicate that the radiolabel accumulates in the thyroid of female rats, that the female rat thyroid contains a metabolite that is not Bromoxynil, and that continuous dosing for two weeks with unlabelled material before administering the radiolabelled material increased the amount of radiolabel that accumulated in the female rat thyroid. Record 139462 is a study that used radiolabelled Bromoxynil Heptanoate. On the surface, record 139462 indicates that there were over a dozen urinary metabolites depending on the sex, dose level and when the urine was collected. Medical Toxicology's discussions of metabolism and related issues can be found in the following documents: W069884.835, W069884.S01 (formerly W075451.S01), R891219, N900808, R910925, R940204 and R950310. That Bromoxynil can act as an endocrine disruptor affecting thyroidal metabolism is discussed at the end of worksheet W168990 833. (Rinkus, 8/11/05).

324-152 139462 "Bromoxynil Heptanoate: Biokinetics & Metabolism Study in the Rat," (Fisher, P.J.; Rhône Poulenc Secteur Agro, Sophia Antipolis, France; study number SA 93027; Dec. 22, 1993). This was a rat metabolism study of Bromoxynil Heptanoate, which is structurally similar to Bromoxynil Octanoate; both esters are active ingredients in the herbicidal product Buctril. The purpose of the study was to provide information concerning the absorption, distribution, metabolism and elimination of Bromoxynil Heptanoate, using compound labelled uniformly with ^{14}C in the phenyl ring. The studies were based on USEPA's 85-1 guidelines. Sprague-Dawley rats (both sexes) were dosed by gavage at 2 or 20 mg/kg in the single-exposure studies and at 2 mg/kg/day for 15 days in the repeated-exposure studies (radiolabelled compound only was administered at the final dosing). Since a major consideration in the 85-1 guidelines is that the metabolites present at $\geq$ 5% of the administered dose be identified using methods that have been validated, the first item that was evaluated was the chromatographic methods. As discussed in worksheet W139462 851, the chromatographic methods (HPLC and TLC) used in the study cannot be considered scientifically sufficient for assigning identities to the metabolites. The most bothersome aspects include the following. In the HPLC analyses, the standards which were tested up to four times exhibited a range of retention times that differed on the order of minutes, depending on the standard. Since not one of the 27 chromatograms regarding urine samples exhibited a metabolite eluting in the interval of 30-40 minutes, there was no HPLC evidence that Bromoxynil was excreted in the urine (the stated retention time of the standard was 34.5 min). Although the report discussed chromatographic results in terms of UMET/1 through UMET/4 (urinary metabolites 1 through 4), analysis of just the first eluting peak in the chromatograms (UMET/1 supposedly) indicated that there were as many as 12 separate metabolites, based on retention times ranging from 10.4 to 19.1 min. There were similar concerns about the TLC data. For example, although five TLC chromatograms were provided, only the two that indicated the start line and the stop line (solvent front) could be checked to confirm the Rf values reported for the standards. In one of the cases, the migration on the plates for the spots that were identified as Bromoxynil Heptanoate and Bromoxynil, Rfs of 0.46 and 0.19, respectively, was not in reasonable agreement with the values reported for the standards, Rfs of 0.51 and 0.22, respectively. Based on the many inconsistencies detailed in worksheet W139462 851, further evaluation of record 139462 has been put on hold pending submission of supplemental information that would validate the HPLC and TLC methods used **Supplemental**

information. (Rinkus, 4/26/05).

324-024 940498 "Bromoxynil Octanoate (M&B 10,731): ^{14}C-Tracer Study of Metabolism and Excretion in Rats" (Ings, R.M.J.; May & Baker, Ltd.; report no. PRG/26; December, 1967). ^{14}C-Bromoxynil Octanoate (labelled in the cyano group) in polyethylene glycol 200 was administered one time orally at 1 or 5 mg/kg to two female Wistar rats/dose/sacrifice time; rats were sacrificed 96, 168 and 336 hours after administration of the radiolabel. **Supplemental information. No worksheet.** (Rinkus, 2/4/94).

324-024 940500 "Fate of Bromoxynil and Carosan Herbicides in Lactating Cows" (Lisk, D.J. et al.; Chipman Chemical Company, Inc., Burlingame, CA; report SR/1/67; 1/23/67). Nonradiolabelled Bromoxynil was mixed with grain and fed to one Holstein cow for four days at the "5 ppm" level. **Supplemental information. No worksheet.** (Rinkus, 2/4/94).

324-034 940499 "Radiocarbon Balance Analysis and Excretion Pattern Following a Single Oral Dose of Bromoxynil, Bromoxynil Butyrate, and Bromoxynil Octanoate in Male Rats" (Thomas, R.D., Kalamaridis, D. and Sexsmith, C.; Borriston Laboratories, Inc.; Borriston Project No. 225-B; 4/26/82). Radiolabelled (^{14}C-label site not specified) Bromoxynil, Bromoxynil Butyrate or Bromoxynil Octanoate in corn oil was administered by gavage one time at 10 mg/kg. Two Sprague-Dawley male rats/sacrifice time/chemical were used; rats were sacrificed 24 and 96 hours after administration of the radiolabel. **Supplemental information. No worksheet.** (Rinkus, 2/4/94).

324-084 055495 "Metabolism of ^{14}C-Bromoxynil Octanoate in a Lactating Goat" (Huhtanen, K.L. and Delaney, P.M.; Union Carbide Agricultural Products Company, Inc., Research Triangle Park, NC and Borriston Laboratories, Inc.; Union Carbide Project No. 854C51; Borriston Project No. 225-C; 8/30/82). ^{14}C-Bromoxynil Octanoate (labelled in the phenyl group) was administered daily in gel capsules for 10 days to a single female Nubian goat. Each capsule contained 4 mg of Bromoxynil Octanoate, which was said to approximate a daily dose of "1 ppm in the diet." The goat was sacrificed 24 hours after the 10th dosing. **Supplemental information. No worksheet.** (Rinkus, 2/4/94).

324-084 055496 "The Biokinetics and Metabolism of ^{14}C-Bromoxynil Octanoate in Rats" (Hawkins et al.; Huntington Research Centre, England; HRC Report No. M&B 181/8448; 2/24/84). ^{14}C-Bromoxynil Octanoate (labelled in the phenyl group) in polyethylene glycol 400 was administered once by gavage at 2 and 20 mg/kg to 5 CD rats/sex/dose; rats were sacrificed 168 hours after administration of the radiolabel. For a whole-body autoradiography study, 5 CD female rats were gavaged with radiolabelled Bromoxynil Octanoate at 20 mg/kg and were sacrificed at 7 hours, 1 day, 3 days, 7 days and 14 days after administration of the radiolabel. **Supplemental information. No worksheet.** (Rinkus, 2/4/94).

324-084 055497 "Investigation of the Metabolites of ^{14}C-Bromoxynil Octanoate in Rat Tissues" (Hawkins, D.R., Elsom, L.F. and Girkin, R.; Huntington Research Centre, England; HRC Report No. M&B 192/84310; 5/10/84). ^{14}C-Bromoxynil Octanoate (labelled in the phenyl group) in polyethylene glycol 400 was administered once by gavage at 20 mg/kg to 6 CD female

rats/sacrifice time; rats were sacrificed 3 days and 7 days after administration of the radiolabel. **Supplemental information. No worksheet.** (Rinkus, 2/4/94).

324-136 115688 "Nature of the Residue Study with ^{14}C-Bromoxynil Octanoate in Dairy Goats" (Downs et al.; PTRL East, Inc.; Laboratory Project No. 610; 6/5/92). ^{14}C-Bromoxynil Octanoate (labelled in the phenyl group) was administered orally using gelatin capsules twice daily for 7 consecutive days to two female goats. Capsules contained sufficient Bromoxynil Octanoate to approximate a daily dose of "10 ppm in the diet;" this was equivalent to a dose of 2.67 mg/kg/day. The goats were sacrificed 13-14 hours after the last dosing. **Supplemental information. No worksheet.** (Rinkus, 2/4/94).

324-137 115691 "Nature of the Residue Study with ^{14}C-Bromoxynil Octanoate in Laying Hens" (Downs et al.; PTRL East, Inc.; Laboratory Project No. 611; 6/5/92). ^{14}C-Bromoxynil Octanoate (labelled in the phenyl group) was administered orally using gelatin capsules twice daily for 7 consecutive days to 10 laying hens. Capsules contained sufficient Bromoxynil Octanoate to approximate a daily dose of "10 ppm in the diet;" this was equivalent to a dose of 6.5-6.8 mg/kg/day. The hens were sacrificed 13-15 hours after the last dosing. **Supplemental information. No worksheet.** (Rinkus, 2/4/94).

324-147 125665 "(^{14}C)-Bromoxynil Octanoate: Absorption, Metabolism, Distribution and Excretion Following Repeat Oral Administration to the Rat" (D'Souza, G.A.; Hazleton UK; HUK Report No. 198/63-1011; 6/29/93). Nonradiolabelled Bromoxynil Octanoate in polyethylene glycol 400 was administered by gavage for 14 consecutive days at 2 mg/kg, followed by a final dosing using ^{14}C-phenyl-labelled material. Five Crl:CD(SD)BR rats/sex were used; rats were sacrificed 168 hours after administration of the radiolabel. **Supplemental information. No worksheet.** (Rinkus, 2/4/94).

III. TOXICOLOGY SUMMARY: BROMOXYNIL STUDIES

COMBINED CHRONIC TOXICITY & ONCOGENICITY, RAT

324-026, 027, 028 940486, 940487, 940488 Title: Evaluation of the Oncogenic Potential and Chronic Toxicity effects of Technical Bromoxynil in Fischer 344 Rats. (Food and Drug Research Labs, Inc., 1-8-82, Lab No. 5815) Bromoxynil technical grade material, production no. #16391 (no purity stated) - this is the same production no. as in the mouse study below where the material is identified as the phenol, 97% - the two studies overlap in time for in-life portions; doses of 0, 10, 30, 100 ppm given to groups of 60/sex Fischer 344 rats in feed (means over course of the study were 0, 11.4, 33.3 and 118 ppm from analysis of diet, FDRL Study No. 6364). No clinical observation noted at any dose. Suggestion of some chronic toxicity with increase in liver weight at 12 months but not at term with no histopathological finding in liver. An increased incidence in adrenal medullary hyperplasia was found in high dose males over control males but controls had a higher incidence of medullary neoplasms, which differed from hyperplasia on basis of absence of mitotic figures and failure to compress adjacent tissue and

formation of discrete tumor nodules in the hyperplasia. There was no dose-related trend in either finding. No such finding was noted in the females. NOEL = 100 ppm (HDT). No evidence for an oncogenic effect. Initially reviewed as having a possible adverse chronic effect but rereview finds that the liver weight effect was not confirmed histologically and the adrenal effect is not clear in view of the pathological findings as discussed above. Initially reviewed acceptable by JR(G), 3-13-85 and 5-4-87, the study subsequently was downgraded to unacceptable/upgradeable by Gee and Rinkus (W940486.S01) because the selection of 100 ppm as the HDT needed to be justified. The study is now considered **UNACCEPTABLE/NOT UPGRADEABLE** because records 075453, 086574 and 069884 indicate that rats can tolerate exposure for 13-104 weeks to $\geq$ 600 ppm Bromoxynil in the diet. Therefore, the selection of 100 ppm as the high dose in this study is not justified as a reasonable approximation of a maximum tolerated dose. (Rinkus 2/16/95).
EPA: Core supplementary for both chronic and oncogenicity. According to the EPA status report, dated December, 1984, Document 324-070, Record # 10422, there was a data gap for rat oncogenicity at that time. No discussion of any studies.

324-034 940484 Duplicate of 940486 without appendices.

****50687-013 069884** "Combined Chronic Toxicity and Oncogenicity Study with Bromoxynil Phenol in Rats", (Hazleton Laboratories America, Inc., study no. 400-712, 3/24/88), bromoxynil (3,5-dibromo-4-hydroxybenzonitrile), 98.5% purity, fed in the diet nominally at 0, 60, 190, or 600 ppm for 104 weeks with 105 (control) and 70 Sprague-Dawley rats/sex/group. No oncogenicity reported. Increased incidence of eosinophilic cellular alteration and spongiosis hepatis in livers of 600 ppm males. Elevated levels of alanine aminotransferase reported at week 105 in 190 and 600 ppm males. Nominal NOEL = 60 ppm (increased serum alanine aminotransferase in males). When first reviewed in 10/7/88, an adverse-effect identification was not made and the study was considered unacceptable but upgradeable pending submission of: (1) subchronic data justifying selection of highest treatment level; (2) information on method of analysis of diet preparations; and (3) justification of testing unesterified form for registration of esterified forms of Bromoxynil. Item 2 was considered satisfied on 10/23/89 by the submission of record 075454. Item 1 is now considered satisfied by the submission of record 086574. Item 3 has been nullified by the decision to accept the USEPA determination that Bromoxynil and Bromoxynil Octanoate are toxicology equivalent (discussed on p. 2 of the Summary of Toxicology Data dated 3/10/95). Upon reconsideration, the nonneoplastic liver lesions (eosinophilic cellular alteration & spongiosis hepatis) and increased serum aminotransferases seen in the males are now considered a possible adverse effect, with a **NOAEL = 60 ppm (increased incidence of spongiosis hepatis in the males)**. Items 1 and 3 and the new adverse-effect identification are discussed in worksheet W069884.S02. This study is now considered **ACCEPTABLE**. (Rinkus, 3/10/95).

324-108 075454 "Determination of Bromoxynil Phenol in Rodent Feed," (Hazelton Laboratories America, Inc.; Analytical Method 105, dated 10/1/84; revised 5/14/85 & 12/23/86). This submission, which was actually an appendix to the original report, adequately explains the method of analysis used in record 069884. (Rinkus, 10/23/89; Rinkus, 3/10/95).
324-108 075451 "Review of the Animal Metabolism of Bromoxynil, Bromoxynil Butyrate, and

Bromoxynil Octanoate" (Huhtanen, R.W. [author]; Rhone-Poulenc Ag Co.; Project ID No. 854R14/file no. 40029; 5/7/87). This record is a review of results presented in records 940499, 055495, 055496, and 055497 and in a study not on file presently at CDFA, entitled "Residue Transfer to Tissues and Milk in Dairy Cows Treated with Bromoxynil Octanoate." This record was submitted as justifying the use of toxicological studies done with unesterified Bromoxynil in the process to register Bromoxynil Octanoate. The inadequacies of the metabolism studies presently on file at CDFA were discussed in the 10/7/88 review of record 069884. (Rinkus, 11/3/89; Rinkus 3/10/95).

SUBCHRONIC, RAT

324-108 075453 "Bromoxynil Technical: 13 Week Toxicity Study in Rats by Dietary Administration" (Research Laboratories of May & Baker; R. Tox 245; July, 1983). Bromoxynil was administered in the diet for 13 weeks at 0, 30, 150, and 600 ppm, to 15 CD rats/sex/treatment group. No deaths or clinical signs were considered related to treatments. Bodyweight effects were limited to the high-dose females, which after 13 weeks showed 21% lower bodyweights than the controls. After 12 weeks of dietary exposure, the following blood chemistry effects were seen: urea nitrogen and alkaline phosphatase were increased in high-dose females; albumin was increased and total bilirubin were decreased in high-dose rats of both sexes; and glucose was decreased in high-dose males. At sacrifice, the following organ weights relative to BW were increased: liver, kidney, and brain in high-dose rats of both sexes; heart in high-dose females; and liver in mid-dose females. However, histological studies did not identify any lesions that could be related to the treatments. **Supplemental information.** (Rinkus, 10/25/89).

324-108 075452 This record is a brief synopsis of the results presented in record 075453. No worksheet. (Rinkus, 10/24/89).

324-111 086574 "Subchronic Toxicity Study in Rats--Bromoxynil" (Hazleton Lab-oratories America, Inc.; Project No. 400-711; November 1, 1985). Bromoxynil was administered in the diet for 13 weeks at 0, 400, 755, and 1456 ppm to 15 Sprague-Dawley rats/sex/treatment group. Survival of both sexes was affected by the high dose. Fourteen females in the high-dose group were found dead on test days 4-5; and the lone survivor of this group was moribund sacrificed on day 24. Each of the high-dose females found dead had lost 11-39 grams in bodyweight since the study started; but apparently, they did not show otherwise any clinical signs that would be suggestive of their impending deaths. Seven high-dose males were found dead on test days 5-10; three were moribund sacrificed on test day 9; but the other 5 members of this group survived to the end of the study. Each of the high-dose males found dead or moribund sacrificed had lost 30-54 grams in bodyweight since the study started; but four of the high-dose males found dead apparently did not show otherwise any clinical signs before their deaths. For the surviving rats, bodyweights measured at weeks 4, 8 and 13 were affected in a dose-response fashion in both sexes: mid- and high-dose males had mean BWs that were 75-86% and 37-50% of the control values, respectively; and low- and mid-dose females had mean BWs that were 88-91% and 78-81% of the control values, respectively. These effects on bodyweight were not accompanied

by a clear decrease in food consumption, except in the case of the high-dose male group. The following hematological effects were seen: decreased platelet counts (high-dose male and mid- and low-dose female groups at week 6; and high-dose male and mid-dose female groups at week 13); increased hemoglobin concentration and (or) increased hematocrit (mid-dose female group at week 6; and high-dose male group at week 13); and increased WBC (mid-dose female group at week 6). The following blood chemistry effects were seen at week 13: an increase in serum chloride in the high-dose male and low- and mid-dose female groups; a decrease in total serum protein in the high-dose male group; an increased albumin/globulin ratio in the high-dose male and low- and mid-dose female groups; increased BUN in the high-dose male group; increased SGOT and SGPT in the mid- and high-dose male groups; and increased serum alkaline phosphatase in the mid- and high-dose male and the mid-dose female groups. Comparable blood-chemistry effects were seen also at week 6 for the following endpoints: total serum protein, albumin/globulin ratio, BUN, SGOT, SGPT, and serum alkaline phosphatase. Testing at week 6 also indicated the following: decreased serum potassium in the high-dose male and mid-dose female groups; increased serum albumin in the low- and mid-dose female groups; increased total bilirubin in the high-dose male group; and decreased serum glucose in the mid- and high-dose male groups. Gross necropsy observations in several organs were found not to be associated with any lesions when examined histologically. However, two gross necropsy effects were not subject to histological examination. Dark red or brown adrenals (bilateral) were noted in 4 high-dose males that were found dead or sacrificed moribund on test days 9-10; and dark foci or areas in the stomach were observed in the following: 6 of the high-dose males that were found dead; one of the high-dose males that were moribund sacrificed; 3 of the high-dose males that survived till the end of the study; the 14 high-dose females that were found dead; 2 of the mid-dose females; and 2 of the low-dose females. A variety of organs in the mid- and (or) high-dose male groups had absolute weights that were reduced and organ/BW ratios that were increased. In the females, only the pituitary had an absolute weight that was reduced for the mid-dose group; but, organ/BW ratios for many organs from the low- and (or) mid-dose groups also were increased in comparison to the corresponding control values. Given their correlation with depressed bodyweights in these groups, these effects do not appear to be indicative of a toxicological effect per se on these organs. Also, the only histological finding was testicular atrophy or degeneration seen in the testes of 2 of the high-dose males that were found dead on test days 5 or 9 and 2 of the high-dose males that survived till the end of the study.
Supplemental information. (Rinkus, 10/11/91).

324-023 076160 "Bromoxynil and Bromoxynil Octanoate: Toxicological Studies," (Biggs, R. et al.; May & Baker, Ltd. report no. AD/54/BIOCHEM/301; October, 1964). This record summarizes the existing toxicology data as of 1964 for these compounds. It is notable for its table (p. 7) summarizing acute oral and parenteral LD50 data in a variety of mammalian species and for its discussion of a 3-month feed study in rats using the potassium salt of Bromoxynil. Supplemental information. No worksheet. (Rinkus, 1/24/92).

SUBACUTE, RABBIT

324-139 115693 "3-Week Dermal Toxicity Study with Bromoxynil Phenol in Rab-bits"

(Henwood, S.M.; Hazleton Wisconsin, Inc.; Laboratory Project ID: HWI 6224-169; 3/31/92). Bromoxynil was applied neat to the backs of 5 Hra:(NZW)SPF rabbits/sex/group, at 0 (water), 30, 300 and 1000 mg/kg, for 6 h/day, 5 days per week, for 3 weeks. The area of exposure was ~10% of the total body surface area (actual dimensions were not provided); and was covered during the 6-h exposure period. **Supplemental information; no worksheet.** (Rinkus, 12/18/92).

CHRONIC, DOG

50687-012 69883, "Bromoxynil--Oral Toxicity in Beagle Dogs Repeated Daily Dosage for 52 Weeks", (Huntingdon Research Centre Ltd., UK; study # M & B/248, 7/1/88), bromoxynil, purity and stability not provided, administered orally by gelatine capsule at 0 (lactose), 0.1, 0.3, 1.5, or 7.5 mg/kg/day with 6 Beagle dogs/sex/group for 52 weeks. **No adverse effects reported. NOEL = 0.3 mg/kg/day (increased absolute liver weight and decreased body weight gain). This study was considered originally by Green & Rinkus (10/6/88) as unacceptable, but upgradable pending submission of: 1) test article purity and stability; 2) content analysis; and 3) justification of testing unesterified form for registration of esterified forms of bromoxynil. Items 1 and 2 were satisfied on 10/20/89 with submission of record 075449. Item 3 has been nullified by the decision to accept the USEPA determination that Bromoxynil and Bromoxynil Octanoate are toxicologically equivalent (discussed on p. 2 of the Summary of Toxicology Data dated 3/10/95). This study is now considered **ACCEPTABLE**. (Rinkus, 3/10/95).

324-108 075449 "1-Year Repeat Dose Oral Toxicity Study in Dogs (Study M&B/248-G) conducted at Huntingdon Research Centre--Associated Analytical Studies" (Buddle et al.; D. Ag. 866). This record is the specific analytical report requested in the 10/6/88 review of record 069883. These analytical data satisfy the originally identified deficiency for such data. (Rinkus, 10/20/89; Rinkus, 3/10/95).

SUBCHRONIC, DOG

324-152 139458 "Bromoxynil Preliminary Oral Toxicity Study in Beagle Dogs," (Harling R.J., et al.; Huntingdon Research Centre, Huntingdon, England; study number M&B 245/861692; Feb. 25, 1988). Bromoxynil (98.6% purity), in gelatin capsules without any vehicle, was administered orally to beagle dogs (both sexes) 7 days/week for up to 13 weeks as part of a range finding study to find acceptable dose levels for an one-year exposure study. The testing was done in three phases. In the first phase, 12 animals/sex were allocated to the following groups at 2/sex/group: 0, 5, 20, 30, 40 and 50 mg/kg/day. Due to severe toxicity at 30-50 mg/kg/day, their testing had to be stopped after only one or two days; also, toxicity at 20 mg/kg/day limited their dosing to 11-17 days. In the second phase, 7 survivors from the first phase (2 males and 5 females that had been dosed at 30, 40 or 50 mg/kg/day) were combined with five previously untreated animals to form the following groups: 8, 12 and 16 mg/kg/day. The 8 and 12 mg/kg/day groups were dosed for 13 weeks whereas the animals in the 16 mg/kg/day group were

dosed only for 14 days (one female), 29 days (one male) or 42 days (one/sex). In the third phase, four previously untreated animals (2/sex) were dosed at 1 mg/kg/day for 13 weeks. The following discussion of possible treatment-related effects was not based on statistical analyses of the data, due to the small group sizes for both sexes. Three clinical signs were associated with treatments: panting (LOEL = 5 mg/kg), salivating (LOEL = 12 mg/kg), and vomiting (LOEL = 20 mg/kg). Increased rectal temperature was a treatment-related finding (LOEL = 12 mg/kg). The last rectal temperatures recorded before the 5 deaths that occurred in the 30-50 mg/groups ranged from 40.0-42.4°C (104.0-108.3°F); therefore, increased rectal temperature of this degree seemed predictive of death in the acute setting. Also, increased rectal temperatures of a lesser degree seen in the first days of dosing (39.2-40.4°C [102.6-104.7°F]) seemed predictive of a moribund condition in the subacute setting. Body-weight gain was reduced in each of the Bromoxynil-treated groups; the reduced body-weight gain in the 1-12 mg/kg groups occurred in the absence of a decrease in feed consumption, which was seen only at doses of $\geq$ 16 mg/kg. Decreases in the following hematological endpoints were noted in the animals dosed at $\geq$ 8 mg/kg: packed cell volume, hemoglobin concentration and red blood cell count. Possibly, four serum-chemistry endpoints were affected by treatment when measured in test weeks 13 and (or) 6. Reduced alkaline phosphatase was observed at both times in the males dosed at $\geq$ 1 mg/kg and in the females dosed at $\geq$ 8 mg/kg. Increased blood urea nitrogen (BUN) was seen at both times in males dosed at $\geq$ 8 mg/kg whereas increased BUN was observed at test week 6 in females dosed at $\geq$ 8 mg/kg but an increase at 8 or 12 mg/kg at week 13 in the females was doubtful. Serum calcium appeared to be reduced at week 13 in both sexes dosed at 12 mg/kg. Serum phosphate tended to be reduced at week 13 in both sexes dosed at $\geq$ 8 mg/kg. Based on increased relative organ weight in the presence of significantly reduced body-weight gain, the liver and kidneys in both sexes and the adrenals in the females underwent a hypertrophic response at doses of $\geq$ 5 mg/kg. Also, testicular atrophy was observed in males dosed at $\geq$ 8 mg/kg. No treatment related changes were observed at necropsy nor histology; however, the histological examinations were limited to gross lesions, kidneys, liver, thyroids and parathyroids with one exception: all major organs were examined histologically in the 16 mg/kg group.
NOEL < 1 mg/kg (reduced body-weight gain and reduced alkaline phosphatase in males at weeks 6 and 13). Supplemental information. (Rinkus, 10/26/04)

ONCOGENICITY, MOUSE

Note: The finding of hepatocarcinogenesis in records 940489/940490 has been corroborated in record 130427. The latter also indicates that both sexes are affected (with the male LOAEL being significantly less than the female LOAEL) and that at least in males the liver cancer occurred at treatment levels that did not produce nonneoplastic effects in the liver. (Rinkus, 3/10/95).

****324-029, 030 940489, 940490** Title: Evaluation of Oncogenic Potential of Bromoxynil Administered in the diet to Swiss Albino Mice for 18 Consecutive Months. (Food and Drug Research Labs, Inc., 12-31-80, Report No. 5798) Bromoxynil phenol, Technical grade,

Production No.16391, about 97% pure; doses given to mice in feed at 0, 10, 30, and 100 ppm (actual mean levels from diet analyses were 12.8, 37.7 and 118 ppm); 60/sex/group. Increased relative and absolute liver weights in high dose males and females - increase in males was associated with increased incidence of hepatocellular proliferative lesions (hyperplastic nodules and tumors) but no histopathological findings in females (metabolic response); increase in kidney weights in females but no histopathology.

Incidence of liver findings in MALES

Dose (ppm)	0	10	30	100
Adenoma, hepatocellular	2/47	4/48	3/49	5/53
Carcinoma, "	0	0	2	4
Tumors, total	2 (4%)	4 (8%)	5 (10%)	9 (17%)

Report states no statistically significant differences in regards to tumors but a statistically significant increase by trend analysis. Comparison of high dose with control by Fisher's Exact test yields a value of 0.034. Report states the incidence of hepatocellular tumors in the concurrent control males was "randomly low" and dismisses the biological significance of the finding. No data to support the statement are included in the report. Acceptable. Reviewed by JR(G), 3-12-85.

324-011 940485 Summary of the above.

324-034 940483 Duplicate of the above only no appendices.

****51644-003 130427** "Oncogenicity Study with Bromoxynil Phenol in Mice," (K.D. Williams; Hazleton Wisconsin, Inc., Wisconsin; Project Identification Number: HWI 6224-174; May 9, 1994). Bromoxynil, 94.4% purity, was administered in the diet for 78-80 weeks at 0, 20, 75 and 300 ppm to 60 Crl:CD-1®(ICR)BR VAF/Plus mice/sex/treatment group. Dose levels were chosen on the basis of a 12-week dietary study, record 118983. Survival at 78 weeks for all treatment groups (both sexes) was $\geq$ 60%. The only bodyweight effect was an increase observed with the 300 ppm female group during test weeks 4 to 42. At week 53, the 300 ppm groups (both sexes) exhibited increased hematocrits. At week 79, each of the male groups exposed to Bromoxynil showed increases in RBC counts, hemoglobin concentration, and hematocrit; significant decreases in WBC counts and neutorphils also were seen in the 300 ppm male group. The 300 ppm groups (both sexes) exhibited significant increases in the absolute weights of the liver/gall bladder and kidneys. Both nonneoplastic and neoplastic lesions were induced in the liver by treatments. The former included: centrilobular hypertrophy, generation/necrosis, and pigmentation of Kupffer cells and hepatocytes. **NOAEL (nonneoplastic liver effects) = 20 ppm.** The combined incidence of adenomas plus carcinomas were significantly elevated in each of the male groups exposed to Bromoxynil (0 ppm: 5/60; 20 ppm, 16/60; 75 ppm, 12/60; and 300 ppm, 23/60) and in the 300 ppm female group (0 ppm, 2/60; 300 ppm, 7/59). **NOAEL (neoplastic liver effects) = <20 ppm.** This study is considered **ACCEPTABLE**. (Rinkus, 2/8/95).
51644-002 112055 This record is a protocol for a second mouse (CD-1) oncogenicity study of Bromoxynil. Proposed dose levels are: 0 (feed), 20, 75, and 300 ppm. **Supplemental**

information. No worksheet. (Rinkus, 1/24/92).

SUBCHRONIC, MOUSE

324-143 118983 "Subchronic Toxicity Study with Bromoxynil Phenol in Mice" (Williams, K.D.; Hazleton Wisconsin, Inc.; Laboratory Project ID: HWI 6224-167; 10/12/92). Bromoxynil was administered in the diet at 0, 10, 30, 100, 300, and 1000 ppm for 12 weeks to 10 Crl:CD-1®(ICR)BR mice/sex per treatment level. A seventh group whose diet contained 3000 ppm Bromoxynil did not survive past the first week of dosing. **Supplemental information. No worksheet.** (Rinkus, 12/18/92).

REPRODUCTION, RAT

324-025 940497 Title: Evaluation of the Effects of Bromoxynil on the Reproductive Performance of FDRL Wistar Rats Through Three Successive Generations. (Food and Drug Research Labs, Inc., 9-20-78, Lab Report No. 5096) Bromoxynil, Technical grade material, [stated as 97%, phenol, #16391, in Record #51068]; groups of 10 males and 20 female Wistar rats given 0, 30, 100, or 300, ppm in feed for 100 days before mating F_0 animals. Three generations, two litters each. NOEL = 30 ppm (decreased weight gain in weanlings and pups.) Fetotoxicity seen in several generations at high dose but not consistent finding. Decreased weight gain at 100 and 300 ppm throughout study. Unacceptable (no analyses of diet for actual content), possibly upgradeable. Reviewed by JR(G), 3-12-85 and 5-5-87.

50687-003 51068 Supplement to 940497. Analysis of technical material used in 940497. [Note: The tolerance number, 50687, is for the butyric acid ester. There is no number for the phenol, the form actually used in the study.]

50687-004 51069 Supplement to 940497. Individual body wts and food consumption with Anovas, and Anovas on pup wts for 940497 - duplicate of data in 940497.

50687-005 51070 Supplement to 940497. Necropsy and histopathology data for F2b adults for 940497.

50687-006 51071 Supplement to 940497. Lab book pages of individual data for 940497.

324-011 940496 Abstract of the above, no data.

****324-106 075104** "Bromoxynil: Effects upon Reproductive Performance of Rats Treated Continuously throughout Two Successive Generations," (Life Science Research, England; LSR Report 89/0343; June 20, 1989). Bromoxynil, purity of ~98%, was presented in the feed at concentrations of 0, 10, 50, and 250 ppm, that were verified analytically. F0 rats were exposed for 14 and ~22 weeks before their 1st and 2nd matings, respectively, and were exposed for a total

of 31-34 weeks before they were sacrificed. F1 rats, derived from the F1a litters, were exposed for 14 weeks before their only mating and were exposed for a total of 23-25 weeks before they were sacrificed. F0 parental effects were limited to the high-dose group and included: 7% lower BWs before the 1st mating & at terminal sacrifice for females only; 5-10% lower gestational & post partum BWs for both pregnancies; and increased relative liver weights (both sexes). F1 high-dose dams and males weighed 15-19% less than controls after weaning; both of their BWs were still lower, by 6-9%, at mating and at terminal sacrifice; F1 high-dose dams also had 3-9% lower gestational & post partum BWs. F1 high-dose rats had increased relative kidney weights (both sexes) and relative liver weights (females only). Fertility was not affected by treatments. **Parental NOEL = 50 ppm (reduced bodyweight, increased relative liver & kidney weights). Parental NOAEL = ≥ 250 ppm.** Progeny effects were limited to the high-dose groups and included: lower post partum BWs; delayed onset & completion of eye opening; and increased incidence of hydronephrosis and/or hydroureter in terminated weanlings. **Progeny NOEL = 50 ppm.** This study was originally classified (Rinkus, 9/13/89) as unacceptable but upgradable upon submission of the following: 1) justification for testing unesterified form for registration of octanoate; 2) F1 histological data for ovaries, seminal vesicles, kidneys, & liver; 3) adequately described historical control data for pup incidence of hydronephrosis & hydroureter; and 4) explanation of why F1 uterine weights were not determined. Items 2-4 were considered resolved on 10/23/91 by submission of records 087040, 095805 and 091873. Because of the nature of the historical control data in record 091873, the designation of hydronephrosis/hydroureter in terminated weanlings was dropped as a possible adverse effect for this study. Item 1 has been nullified by the decision to accept the USEPA determination that Bromoxynil and Bromoxynil Octanoate are toxicologically equivalent (discussed on p. 2 of the Summary of Toxicology Data dated 3/10/95). This study is now considered **ACCEPTABLE**. (Rinkus, 3/10/95).

324-114 087040 This record contains tables that appeared in record 075104 now corrected for mislabelling regarding the uterus examination of the F0 females and for omission of certain histopathology data regarding the ovaries and seminal vesicles. No worksheet, but discussed briefly in the worksheet W075104.S01. (Rinkus, 10/23/91).

324-117 095805 This record contains new data for record 075104. These data concern the histological examination of the liver and kidneys in the members of control and high-dose groups in the F0 and F1 generations. No worksheet, but discussed briefly in the worksheet W075104.S01. (Rinkus, 10/23/91).

324-119 091873 This record consists of tables without explanatory text that concern the historical negative-control data regarding hydronephrosis and hydroureter at the contract laboratory that performed the study in record 075104. No worksheet, but discussed briefly in the worksheet W075104.S01. (Rinkus, 10/23/91).

TERATOLOGY, RAT

Note: The open literature contains two publications regarding oral-exposure, developmental-toxicity testing of Bromoxynil Octanoate and Bromoxynil. In Rogers et al. ("Developmental Toxicity of Bromoxynil in Mice and Rats," Fund. Appl. Toxicol. 17:442-447, 1991), Bromoxynil Octanoate was tested using rats while Bromoxynil was tested using mice and rats. In Chernoff et al. ("Significance of Supernumerary Ribs in Rodent Developmental Toxicity Studies: Postnatal Persistence in Rats and Mice," ibid., p. 448-453), the postnatal fate of supernumerary ribs induced by Bromoxynil in mice versus rats was studied. Full reports of these two studies have not been submitted to DPR (note: the submission of these to DPR should include all of the individual data). (Rinkus, 8/11/05).

324-161 168990 "3,5-Dibromo-4-Hydroxybenzoic Acid (DBHA): Developmental Toxicology Study in the Rat by Gavage," (Foulon, O.; Rhône-Poulenc Agro, Sophia Antipolis, France; study number SA 97401; March 1, 1999). DBHA was tested because it is an expected plant metabolite when plants genetically engineered to express nitrilase enzymatic activity are treated with Bromoxynil or its derivatives. DBHA, 99% purity, was given by gavage on gestation days 6 through 15 to 23-25 pregnant Sprague-Dawley rats per group at 0 (0.5% methylcellulose 400 in water [w/v]), 50, 150, and 500 mg/kg and dams were sacrificed on gestation day 20. (The high dose was selected on the basis of a range-finding study that was not included in the report. Only two results from the range-finding study were mentioned: at 1000 mg/kg, no females had viable fetuses at scheduled sacrifice; and at 600 mg/kg, reduced maternal bodyweight change and lower food consumption from gestation days 6 to 12 were observed.) Maternal effects in the full study were limited. The only clinical sign was increased salivation after gavaging in the 500 mg/kg group; it was noted in 11 of the 25 dams on test, generally on gestation days 13, 14 and (or) 15. Maternal bodyweight gain between gestation days 6 to 8 was decreased statistically ($p \leq 0.01$) in the 500 mg/kg group; this involved 16 of the 25 pregnant dams on test, with 13 of the 16 exhibiting a bodyweight decrease for a single day during this two-day period. However, the importance of this is questionable given that there was no effect on absolute mean bodyweights over this same interval. Mean placental weight in the 500 mg/kg group was increased statistically ($p \leq 0.01$) for both sexes of fetuses. Fetal bodyweights were not affected; for example, both sexes of fetuses from the 500 mg/kg group exhibited mean bodyweights that were 96% of the respective values measured in the negative controls. A significant external fetal finding was made in the 500 mg/kg group: 8 fetuses (involving five litters) exhibited no tail (N=2), thread-like tail (N=4; one of which also had abnormal dorsal flexure of the back) or a tail in an abnormal position (N=2). Skeletal examination indicated that each of the 8 fetuses had findings in the lumbar-sacro-caudal region of the vertebral column. Regarding the remaining fetuses from the study, about half received visceral examinations while the other half received skeletal examinations. The visceral examinations did not find any effect of significance, except possibly an increased incidence of dilated renal pelvis (unilateral and bilateral combined) in the 500 mg/kg group ($p \leq 0.05$). Skeletal findings included statistically increased litter incidences in the following in the 500 mg/kg group: incomplete ossification of the interparietals ($p \leq 0.001$); 27 presacral vertebrae ($p \leq 0.001$); one or more sternebrae misaligned ($p \leq 0.001$); defective thoracic centrums (hemicentric or exhibiting an ossification defect; $p \leq 0.001$); extraossification sites on the 14 thoracic vertebra (unilateral and bilateral combined: $p \leq 0.001$); and a 14^{th} pair of thoracic ribs (short in length) ($p \leq 0.001$). At 500 mg/kg, 33% of the fetuses (62 of 190 examined)

exhibited a supernumerary rib of some type, involving the 14th thoracic vertebra. Several incipient skeletal effects also were suggested at 150 mg/kg; e.g., the incidence of bilateral supernumerary ribs (any type) was increased ($p \leq 0.05$). The **maternal NOEL is >500 mg/kg** (the bodyweight effect at 500 mg/kg is too weak to be considered intoxication). The **developmental-toxicity NOEL is 50 mg/kg** (incipient skeletal effects at 150 mg/kg, with unequivocal skeletal effects, including tail agenesis, at 500 mg/kg). Both NOELs can be reevaluated after review of the range-finding study and the historical negative-control data from the conducting laboratory regarding visceral and skeletal examinations (discussed in worksheet w168990 833). **Supplemental information.** (Rinkus, 7/21/04).

****324-034 940493** Title: Bromoxynil Technical - Teratogenicity study by the Oral Route in the Rat. (May and Baker Ltd., 9-81, Report no. ref. R. Tox. 66.) Bromoxynil (phenol), technical, 97.1% of dry weight, batch LN 556; doses of 0, 5, 15, or 35 mg/kg given to Sprague-Dawley rats by gavage on days 5-17 of gestation, 28/group. Possible adverse effects include dose-related increase in skeletal anomalies, pre-implantation loss and late uterine deaths. At 35 mg/kg/day, 2 fetuses from 2 litters had microophthalmia. One fetus also had kinked radii and ulnae. At 15 mg/kg/day one fetus had bilateral kidney agenesis and open eye. At 5 mg/kg/day, one fetus had hydrocephaly. Skeletal changes at 5 mg/kg were minimal and mostly involved 14th ribs which change is considered by most teratologists to indicate maternal toxicity. NOEL for developmental toxicity less than 5 mg/kg/day. Maternal NOEL = 5 mg/kg/day. Acceptable. Reviewed by JR(G), 3-11-85 and JAP, 4-15-86.

324-035 940495 Duplicate of the above.

324-034 940492 Title: Teratologic Evaluation of Bromoxynil in Rats [In support of Brominal.] (Food and Drug Research Labs, Inc., 1-3-77, Lab no. 5097) Bromoxynil, no purity stated, no description of test article; doses of 0, 1.5, 5.0 or 15 mg/kg/day given to Wistar rats by gavage on days 6-15 of gestation. Dose related increase in wavy ribs at all treatment levels. Possible adverse effects since developmental toxicity seen at levels below that of maternal toxicity. Maternal NOEL > 15 mg/kg/day (HDT). Developmental NOEL < 1.5 mg/kg/day. Unacceptable, no data present on maternal body weight, clinical observations or necropsy. No evidence or statement that MTD was reached. No individual fetal observations. Reviewed by JR(G), 3-11-85 and JAP, 7-28-86.

324-024 940494 Duplicate of the above.

324-011 940491 Summary of the above.

324-080 37327 Duplicate of 940492.

****008 64834** "Bromoxynil Tech Teratology Study in the Rat." (Life Science Research Israel, 2/12/87) Bromoxynil technical, free phenolic form, 95.8%; given by oral gavage at 0, 4.0, 12.5 or 40 mg/kg/day, days 6 - 15, 22 pregnant CD rats of Sprague-Dawley origin per group; maternal food consumption and body weight gain were decreased at 40 mg/kg/day; maternal NOEL = 12.5 mg/kg/day (body weight, food consumption); developmental NOEL = 12.5 mg/kg/day

(lower fetal weight, skeletal anomalies, microphthalmia/anophthalmia); possible adverse effect seen in other studies is confirmed; Acceptable. Gee, 1/20/88.

50687-014 070304 "Developmental Toxicity Study of Bromoxynil Phenol Administered Percutaneously to Cr1:CD(SD)Br Presumed Pregnant Rats." (Argus Research Laboratories, PA; Argus protocol 310-003; 9/20/88). Bromoxynil, purity not stated and tested as the sodium salt, was applied topically to the backs of 23 mated Sprague-Dawley rats/group, at the nominal levels of 0 (20% aqueous triethylene glycol), 5, 10, 50, and 100 mg/kg/day, about 6 h/day, on days 6-15 of gestation, with sacrifice on day 20. HDT was selected based on a range-finding study that also is included in this report. Maternal effects from the treatments were limited to slight decreases in bodyweight gains and feed consumption in the 100 mg/kg/day group, primarily on days 6-9. Maternal NOEL = 50 mg/kg/day. The only fetal effects were increases in the incidence of extra thoracic ribs in the 50 and 100 mg/kg/day groups. Developmental NOEL = 10 mg/kg/day. Major deficiencies in the study include: 1) test material purity, stability, and method of analysis of dosing solutions were not provided; 2) skin site was not covered in any form; and 3) the study tested a substance (sodium salt of unesterified Bromoxynil) that is different from what is being registered for use as a pesticide (Bromoxynil Octanoate). Supplemental information. (Rinkus, 2/2/89).

324-094 070130 This record contains the protocol to record 070304 and a draft copy of the pilot study, which was included as supplemental data in record 070304. No worksheet. (Rinkus, 2/2/89).

50687-017 (No record number). This submission consists of: 1) a letter from Argus Research Laboratories explaining that to avoid "maternal stress," no covering was used in the rat teratology studies done for the Registrant at Argus (records 070304, 071539, & 075250); and 2) an abstract to a mouse study of "maternal stress" done by Chernoff et al. (Teratology 35: 38A, 1987). (Note: Chernoff et al. also reported in a full publication [Teratogen. Mutagen. Carcinogen. 7:241-253, 1987] that "maternal stress" of the type used in the mouse study did not induce cervical and/or extra thoracic ribs in SD rats). No worksheet. (Rinkus, 10/13/89). (It has come to CDPR MT's attention that this volume number no longer exists in the CDPR library, but a copy of this submission is still on file as a SB 950 response. [Rinkus, 1/24/92])

324-132 112054 "A Review and Critique of Developmental Toxicity Safety Evaluation for Bromoxynil," (E. Marshall Johnson & Mildred S. Christian; dated April 21, 1988). This appears to be a critical review commissioned by the Registrant of the oral rat and oral rabbit teratology studies in which the test matertial had been Bromoxynil. **Supplemental information. No worksheet.** (Rinkus, 1/24/92).

TERATOLOGY, RABBIT

324-079 37325 Title: Bromoxynil Technical Teratogenicity Study by the Oral Route in the Rabbit. (May and Baker, 4-83, Report No R. Tox. 219) Bromoxynil, 97.1%, batch MN2822; doses of 0, 15, 30, or 60 mg/kg/day given to New Zealand white rabbits on days 5-20 of

gestation by gavage, 12 to 23 per group. Maternal NOEL = 30 mg/kg/day (decreased weight gain). Developmental NOEL < 15 mg/kg/day. Adverse effects major malformations were noted at all dose levels and were significantly increased at 60 mg/kg/day. These consisted of hydrocephaly, an- or micro-ophthalmia. Others included kidney agenesis, missing long bones, missing digits, anury, fused ribs and fused jaw. The report (pg 61) states that 77/105 fetuses at the high dose have malformations (including minor anomalies). JAP tabulated some major malformations (anury, agenesis of the kidney, hydrocephaly, an- or microophthalmia and limb defects) and found the following number of affected fetuses and litters:

Control	15	30	60 mg/kg/day
1	2/2	1	36 in 9 litters

This compound should go to risk assessment and this study be evaluated with the other teratology studies which also show a small number of fetuses with some of the same defects. These malformations are very rare and in looking at published control values (14,856 rabbit fetuses), the number of fetuses with an- or micro-ophthalmia was 10 and the number of fetuses with limb
defects was 8. This report was not submitted as an adverse effects report and there was a lag of two and a half years in submitting this study to CDFA. Unacceptable, upgradeable. JAP, 5-2-86.

****324-037 1254** Title: Teratology Potential of Bromoxynil Phenol in New Zealand White Rabbits. (Science Applic. Inc. 4-11-84, Report no UNC/SAI 1282017) Bromoxynil Phenol, 95%, was administered to New Zealand white rabbits on days 6-18 of gestation at dose levels of 0, 30, 45, or 60 mg/kg/day, 22-23 per group. The report states that..."Maternal toxicity at the 60 mg/kg level and teratogenic effects at dose levels of 45 and 60 mg/kg body weight". Adverse effects: Evidence for maternal toxicity at 60 mg/kg is based on death with 7 of 23 dams dying during the study. Necropsy findings do not clearly identify that these deaths were related to treatment. There is no effect on maternal weight gain (however only day 6 and 30 weights are presented) at any dose level. There is no effect on number of dead or resorbed fetuses at any dose level. Fetal weights are decreased at 45 and 60 mg/kg. There is a significant increase in the number of fetuses with malformations at 45 and 60 mg/kg/day. The major malformations noted include hydrocephaly and anophthalmia or microophthalmia. The report discusses the occurrence of hypotrophic (runted) fetuses in the treated groups.

Finding	Control	30	45	60 mg/kg/day
#Fetuses/litters	122/20	145/20	145/20	99/13
Mean fetal wt.	53.1	50.7	48.3	44.5
Anophthalmia	0	0	7/3	1
Microophthalmia	0	0	2/2	2/2
An/microophthalmia	0	0	7/3	3/2
Hydrocephaly	0	1	4/2	8/5
Hydro + an/microophthalmia	0	0	3/1	1/1

Maternal NOEL = 45 mg/kg/day. Developmental NOEL = 30 mg/kg/day. Acceptable. Reviewed by JR(G), 3-12-85 and JAP, 5-1-86.

50687-016 072018 "Developmental Toxicity Study of Bromoxynil Phenol Administered Percutaneously to New Zealand White Rabbits." (Argus Research Laboratories, PA; Argus protocol 310-001; 12/13/88). Bromoxynil, purity not stated and tested as the sodium salt, was applied topically to the backs of 20 inseminated New Zealand White rabbits/group, at the nominal levels of 0 (20% aqueous triethylene glycol), 10, 50, and 150 mg/kg/day, for 6 h/day, on days 6-18 of gestation, with sacrifice on day 29. HDT was selected based on a pilot study wherein 100 mg/kg/day was not obviously toxic to the mothers or the fetuses. Maternal effects from the treatments were limited to slightly decreased bodyweight gains in the 150 mg/kg/day group. Maternal NOEL = 50 mg/kg/day. The only fetal effect was an increased incidence in lung agenesis involving the intermediate lobe in the 150 mg/kg/day group. Developmental NOEL = 50 mg/kg/day. Major deficiencies in the study include: 1) test material purity, stability, the method of analysis of dosing solutions, and content analysis of all vehicle-control dosing solutions were not provided; 2) skin site was not covered in any form and the application site was considerably less than 10% of the total body surface area; and 3) the study tested a substance (sodium salt of unesterified Bromoxynil) that is different from what is being registered for use as a pesticide (Bromoxynil Octanoate). Supplemental information. (Rinkus, 2/8/89).

324-095 070131 This record contains the protocol to record 072018 and a draft copy of the pilot study which was included as supplemental data in record 072018. No worksheet. (Rinkus, 2/8/89).

TERATOLOGY, MOUSE

Note: The open literature contains two publications regarding oral-exposure, developmental-toxicity testing of Bromoxynil Octanoate and Bromoxynil. In Rogers et al. ("Developmental Toxicity of Bromoxynil in Mice and Rats," Fund. Appl. Toxicol. 17:442-447, 1991), Bromoxynil Octanoate was tested using rats while Bromoxynil was tested using mice and rats. In Chernoff et al. ("Significance of Supernumerary Ribs in Rodent Developmental Toxicity Studies: Postnatal Persistence in Rats and Mice," ibid., p. 448-453), the postnatal fate of supernumerary ribs induced by Bromoxynil in mice versus rats was studied. Full reports of these two studies have not been submitted to DPR (note: the submission of these to DPR should include all of the individual data). (Rinkus, 8/11/05).

GENOTOXICITY, GENE MUTATION

****324-064 1359** Title: Mutagenicity Evaluation of Bromoxynil Phenol (Marks) in the Mouse Lymphoma Forward Mutation Assay - Revised Final Report. (Litton Bionetics Veenendaal, The Netherlands, 6-82, Report no. 20989) Mouse lymphoma L5178Y cells were exposed to 15.6 to 250 µg/ml bromoxynil phenol (no purity stated) without activation and 3.91 to 62.5 µg/ml with

activation provided by S9 mix. Trial with S9 was not repeated. Test agent not well defined. Increase in mutation frequency with test agent in the presence of S9 at higher concentration. Acceptable. Reviewed by JR(G), 9-4-85.

324-029 37317 Title: Microbial Mutagen Assays with Technical Bromoxynil (Salmonella Typhimurium) (Gibraltar Biological Laboratories Inc., 9-12-77, Report No. GBL 7516) Bromoxynil Technical (no description, no purity); 100 μg per disk with and without S9; Salmonella strains TA1535, TA1537, TA1538, TA98 and TA100 were exposed. No increase in reversion rate is reported. Unacceptable (no confirming trial, no data for -S9 presented although apparently such plates were prepared. Inadequate description of method in terms of timing especial with S9.) Reviewed by JR(G), 2-6-86.

****324-129 098204** "Mutagenicity Test on Bromoxynil Phenol in the CHO/HGPRT For-ward Mutation Assay," (M.A. Cifone; Hazleton Washington Inc.; HWA study no. 12550-0-435; 7/18/91). Bromoxynil (purity 98.4%) was tested for mutagenicity using the HGPRT locus in Chinese hamster ovary K1-BH_4 cells in the absence and presence of a metabolic activation system (commercial S-9, made from Aroclor 1254-induced male Sprague-Dawley rat liver). The highest dose tested was selected on the basis of cytotoxicity observed in preliminary testing, the results of which were included in the report. Only one experiment was performed, involving one culture per treatment level, except for the vehicle control (2 cultures). Exposure to test materials lasted for 4 hours, followed by a 7 day expression period. The final concentrations tested were: 0 (1% v/v dimethyl sulfoxide), 100, 200, 300, 400, 500, 600, 800, 900 and 1,000 μg/ml. Definite precipitation of Bromoxynil in the culture medium was noted at levels $\geq$ 800 μg/ml. The mutant frequency was not increased in testing in the absence of the metabolic activation system. In the presence of the metabolic activation system, cytotoxicity was greater and it precluded the determination of the mutant frequency at the two highest dose levels; but at 500-800 μg/ml and at 100 μg/ml, statistically increased mutant frequencies were observed. The authors of the report discounted these effects based on the lack of a dose response and the fact that the highest observed frequency was not greater than the maximum frequency observed historically in the appropriate controls. However, it remains that a major deficiency in this testing was the failure to conduct a second trial, since without it, the reproducibilty of these statistically significant effects is unknown. This study is considered **ACCEPTABLE.** (Rinkus, 12/11/91; 3/10/95).

324-129 098205 "Mutagenicity Test on Bromoxynil Phenol in the Salmonella/Mammalian-Microsome Reverse Mutation Assay (Ames Test)," (Lawlor, T.E. & Mecchi, M.S.; Hazleton Washington Inc.; HWA study no. 12550-0-401; 5/22/91). Bromoxynil (purity 98.4%) was tested for mutagenicity in the Ames test, using strains TA1535, TA1537, TA1538, TA98 and TA100, in the absence and presence of a metabolic activation system (commercial S-9, made from Aroclor 1254-induced male Sprague-Dawley rat liver). Only one experiment was performed, involving three platings for each treatment level. The test concentrations when no metabolic activation system was used were: 0 (dimethyl sulfoxide), 3.33, 10.0, 33.3, 100, 333 and 1,000 μg/plate; when a metabolic activation system was used, testing at 3.33 μg/plate was not done and testing at 3,330 μg/plate was included. **No mutagenicity was observed with the test material and the expected results were observed with the positive controls

for the tester strains and the metabolic activation system. This study is considered **ACCEPTABLE**. (Rinkus, 12/6/91; 3/10/95).

GENOTOXICITY, CHROMOSOMAL

****324-064 1357** Title: Mutagenicity Evaluation of Bromoxynil Phenol (Marks) in an in vitro Cytogenetic Assay Measuring Chromosome Aberration Frequencies in Chinese Hamster Ovary (CHO) Cells - Revised Final Report (Litton bionetics Veenendaal, The Netherlands, 7-82, Report No. 20990). CHO-WBI cells exposed to 50 to 1000 µg/ml bromoxynil phenol (no purity stated) without S9 and 300 to 1600 µg/ml with S9. Note record no 1360 using a similar protocol but measuring SCE in M2 (as opposed to M1) reported negative findings. Increase in aberrations, especially simple breaks in x-chromosome. Acceptable. Reviewed by JR(G), 9-4-85.

**324-064 1360 Title: Mutagenicity Evaluation of Bromoxynil phenol (Marks) in the Sister Chromatid Exchange Assay With Chinese Hamster (CHO) Cells - Final Report. (Litton Bionetics Inc. Kensington, MD, 6-82, Project No. 20990). Bromoxynil, no purity stated; Chinese hamster ovary cells were exposed to 4.7 to 350 µg/ml test material without S9 and 400 to 900 µg/ml with S9. No increase in SCE's reported. Acceptable. Reviewed by JR(G), 9-3-85.

324-079 37324 Title: Bromoxynil Micronucleus Test in CD-1 Mice. (Inveresk Research International, Scotland, 6-82, Report No. 2302) Bromoxynil, >99% pure, no further description; 5/sex/group given doses of 0, 21.6, 69 or 215.5 mg/kg by oral gavage in 2 doses at 24-hour interval, sacrificed at 6 hours after second dosing. No adverse effect reported. Unacceptable due to protocol. Study is complete. Reviewed by JR(G), 2-6-86.

324-079 37326 Title: Bromoxynil: Dominant Lethal Study in Rats. (Inveresk Research International, Scotland, 7-82, Report No. 2302) Bromoxynil, stated to be > 99% pure, no further description; ten males/group were dosed for 5 days at 0, 3, 9.6 or 30 mg/kg/day by oral gavage, mated 1:2 females. No dominant lethal effects over 10 weeks. Complete, Unacceptable due to animal numbers. Reviewed by JR(G), 2-6-86.

324-133 112596 "Bromoxynil Phenol: Micronucleus Test in Bone Marrow of CD-1 Mice," (Holmstrom, L.M. et al.; Inveresk Research International, Tranent, Scotland; IRI Project No. 751084; Report No. 8227; 7/26/91). Bromoxynil (97.1% purity) was tested for the induction of micronuclei in bone-marrow polychromatic erythrocytes of CD-1 mice of both sexes. Testing involved one-time gavaging of 5 mice/sex/treatment level and sacrificing them at either 24, 48 or 72 hours later. Treatment levels were: 0 (corn oil), 35, 70 and 105 mg/kg. Sacrificing at 48 and 72 hours postdosing was only done with the corn-oil control groups, the 105 mg/kg groups, and the positive control groups. The selection of the high doses was based on LD50 data that were contained in the report. **No induction of micronuclei was observed whereas the negative control and positive control (cyclophosphamide, 80 mg/kg) gave appropriate results. This study is considered **ACCEPTABLE**. (Rinkus, 11/20/92; 3/10/95).

GENOTOXICITY, DNA DAMAGE/OTHER

****324-064 1358** Title: Evaluation of Test Article Bromoxynil Phenol (Marks) in the Bacterial DNA Repair Test- Revised Final Report. (Litton Bionetics Veenendaal, The Netherlands, 4-82, Report no. 20988) Bromoxynil phenol, no purity stated, duplicate plates exposed to test article at 1, 10, 100, 500, 1000, 2500, 5000, 10,000 μg/plate with pol A+ and pol A- E. coli. with and without activation. Strain pol A- showed a greater zone of inhibition of growth than pol A+. Acceptable. Reviewed by JR(G), 9-14-85.

**324-064 1361 Title: Evaluation of Bromoxynil Phenol in the in vitro Transformation of C3H/10T1 8 Cells Assay - Final Report. (Litton Bionetics Veenendaal, The Netherlands, 5-82, Report No 20992) Bromoxynil phenol, no purity stated (RTC 2801 AB-001/18381-003); mouse cells strain C3H/10T1/2C1 8 were exposed to 32.5 and 390 μg/ml, 20 plates per concentration. No adverse effects noted. Acceptable. Reviewed by JR(G), 9-3-85.

**324-064 1362 Title: Evaluation of Bromoxynil Marks in the Primary rat Hepatocyte Unscheduled DNA Synthesis Assay - Final Report. (Litton Bionetics Veenendaal, The Netherlands, 5-82, Report no. 20991) Bromoxynil RTL 2801 AB-001/18,381-008; primary rat hepatocyte UDS, hepatocytes from one male Fischer 344 rat; exposed to 15 concentrations ranging from 1000 to .025 μg/ml; scored 150 cells/concentration. No increase in unscheduled DNA synthesis reported. Acceptable. Reviewed by JR(G), 9-3-85.

NEUROTOXICITY

A potential neurological effect is indicated in the rat reproduction study, record 075104; delayed eye opening was observed in each of the three sets of high-dose weanlings in that study. For example, hydroxyurea also causes delayed eye opening in rat neonates; this effect has been related to its disruption of the cytoarchitecture of the brain (Vorhees et al., Toxicol. Appl. Pharmacol., 50: 267-282, 1979). The possibility that Bromoxynil acting as an antithyroid agent is causing hypothyroidism in the neonates, a proven way to produce neuroanatomic and behavioural changes in neonatal rats, is discussed in the worksheet to record 075451. (Rinkus, 1/24/92).